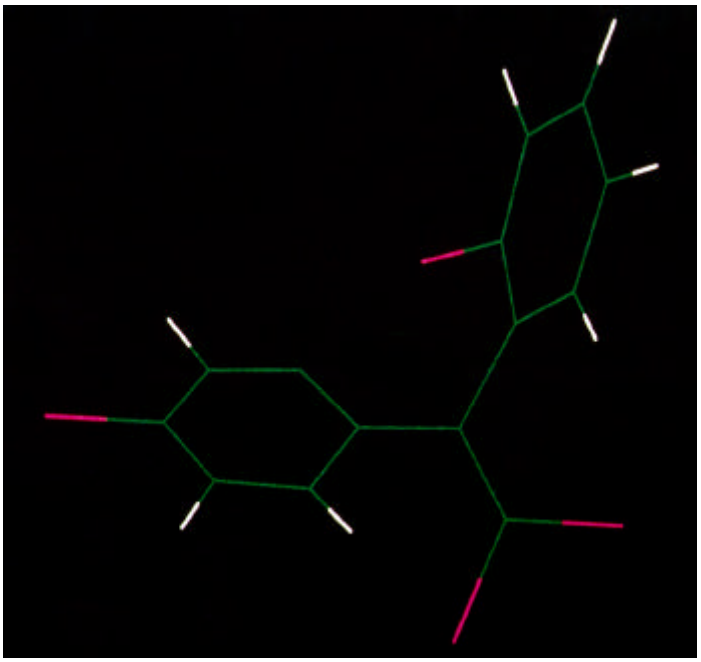
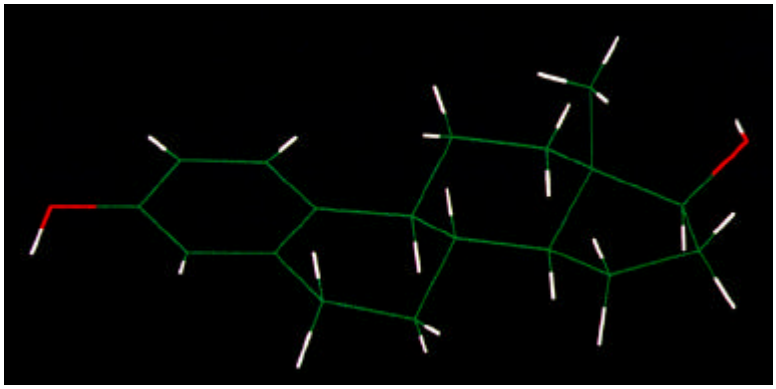
# PCBs, DDT, Breastfeeding, and Growth in North Carolina Children Followed from Birth to Puberty

We have developed evidence over the last fifteen years supporting the hypothesis that specific environmental chemicals act to disrupt some aspects of endocrine function involved in lactation and growth of the child. The evidence comes from studies in which the exposure and the outcome are measured in the same people. While not based on trials, data like these allow inference of a sort not possible by observing secular trends in endocrine related phenomena, which, however interesting and informative, cannot tell you etiology.

I got interested in the presence of industrial chemicals like PCBs and o, p-DDE in breast milk back in the mid-70s. O, p-DDE is a persistent derivative or metabolite of DDT. People enjoyed all but universal exposure to DDT from its introduction in a widespread fashion after World War II until its banning in the United States in 1971.

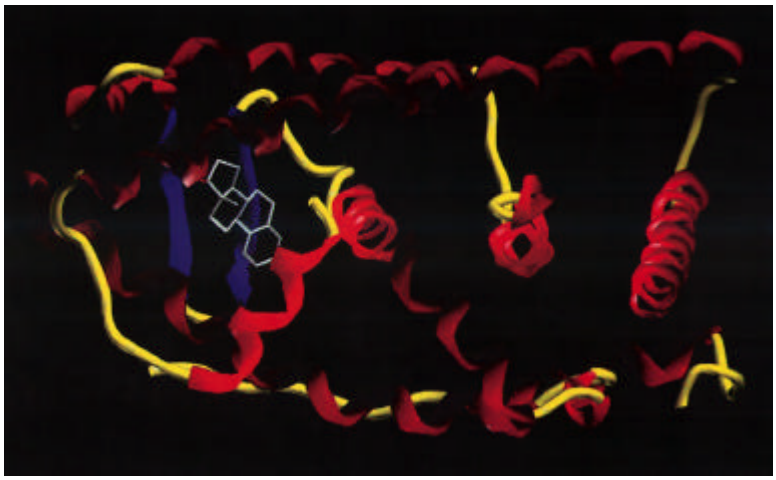
DDT and DDE are very fat soluble and resist environmental or metabolic breakdown. These persistent chemicals were thought to be a big advance in public health practice because you could apply them once and they would stay there. If you were spraying roofs for Chagas' disease vector or you were treating a house for termites, you didn't have to go out and reapply every year or six months with toxic agents like parathion. The legacy of DDT, though, is that since 1951, everybody 's fat and breast milk has residue levels of DDT and DDE.



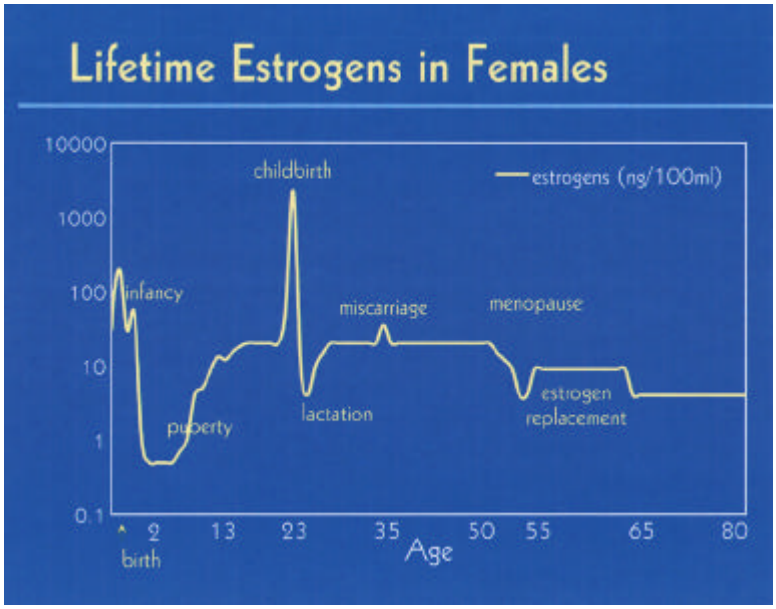


DDE, along with methoxychlor, was one of the first environmental compounds to be shown to be able to occupy estrogen receptors. You can overlay the business end of the 17-beta estradiol

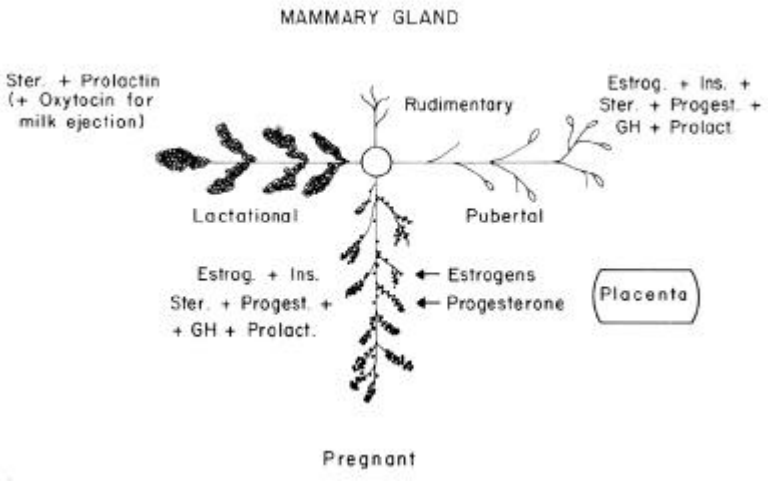
with o,p-DDE, and that will insert into the estrogen receptor



Suppose you want to study whether or not human beings respond to a relatively weak estrogen signal coming from the outside. I would propose to you that the time to study that is not when you have a big endogenous signal, but rather at a time when you have a weak endogenous signal such that you can detect signal over noise. In this case, noise is the normally functioning ovary.  You can map out the levels of estrogen a woman makes over her lifetime,




and look for periods of relatively low endogenous production. Baby girls make estrogen for a while before they go latent. They have very low levels until puberty when they begin to cycle, and then later the levels go up during pregnancy, peaking at around term and then falling abruptly. Normally, estrogen levels at the beginning of lactation are comparable to post-menopausal levels. The very high circulating levels of estrogen during pregnancy are accompanied by very high circulating levels of prolactin. Prolactin is operating to increase the ductal complexity of the breast because milk is synthesized at surfaces.



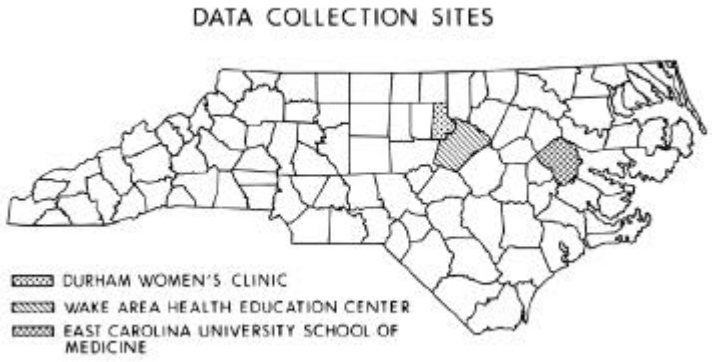

You want to have as much surface area inside the breast as possible in order to synthesize milk. The estrogen is acting to increase the blood supply, the vascularity and the fatty volume of the breast. It is also inhibiting the ability of prolactin to actually promote milk secretion. At term, estrogen falls precipitously, prolactin is acting unopposed and full milk synthesis begins.

Now two decades ago before there were specific anti-prolactin agents like bromocriptine, women who did not wish to breast feed were given a gram or two of diethylstilbestrol in the delivery room. That very big estrogen dose would stop the action of prolactin on the breast and they would cease lactating.

If you propose that there is a weak estrogen signal around when there is not supposed to be one here at term, then you might observe what you observe in the oral contraceptive literature. Women who went on the old-fashioned high-dose estrogen oral contraceptives in the immediate postpartum period didn't make as much milk. Consequently, they didn't breast feed as long. The reason they didn't breast feed as long is because the kid is unhappy and complains. And so they supplement, and once you begin the

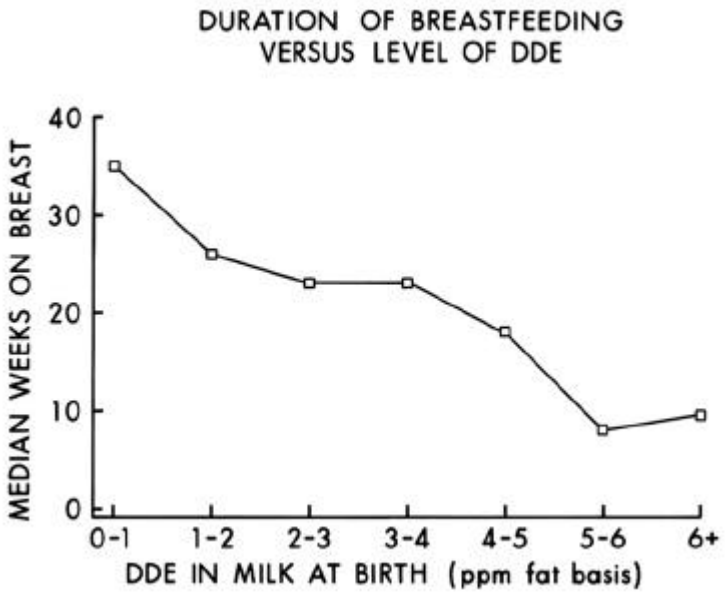
supplementation process the child sucks less aggressively and thus produces a weaker prolactin response signal from mom. So low milk supply early and early supplementation ends up with shorter periods of lactation.

We hypothesized that, if o,p-DDE was acting as a weak estrogen, then we would observe that women with higher DDE levels would not breast feed as long. We tested this in data from a study of 856 children born in North Carolina

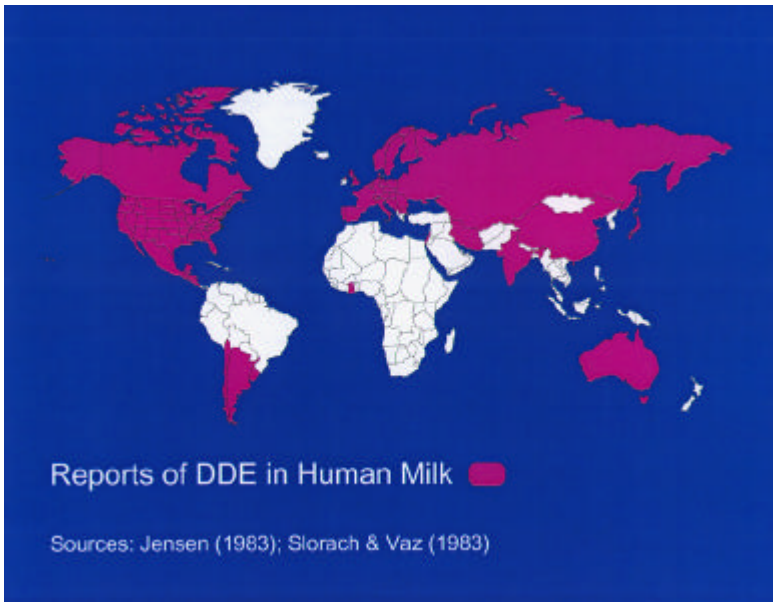



between 1978 and 1982. We were trying to see whether the PCBs and DDE that were present in breast milk could be seen to affect the growth and development and illness history of the kids. We followed those kids developmentally and clinically from birth through five years of age. Then, we followed them by mail.

We had data on duration of lactation. We rate the women by the level of DDE in milk fat at birth. Surprisingly enough to us, our hypothesis was actually borne out. That is, the women who were in the lowest fifteen percent of DDE levels breast fed on the average about thirty-five weeks. The highest ten percent or so of women breast fed on the average about nine weeks. There was a fairly smooth dose-response relationship.

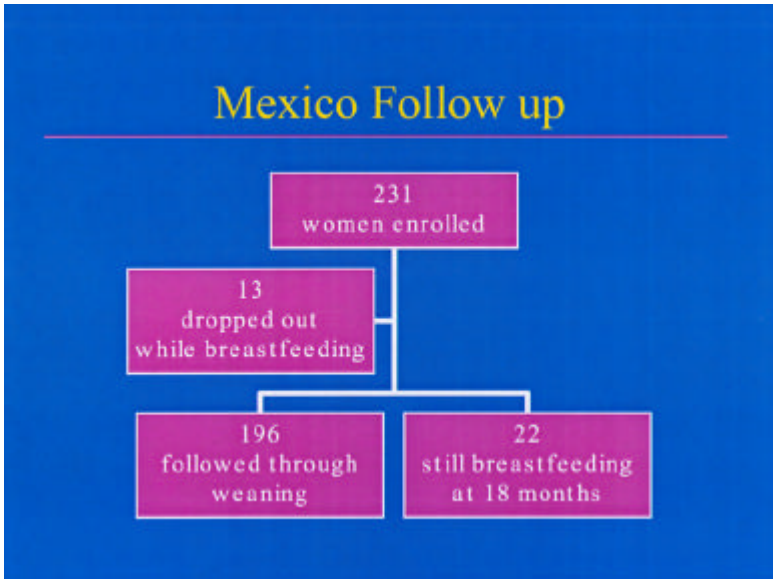



We looked at this also by whether women weaned for reasons other than would be plausibly related to an estrogen: Women who had to go back to work, women who got an infection, women who were put on a drug that their physician thought would not be a good idea to have the kid exposed to. We split the women into reasons like that and reasons like insufficient milk. The relationship held only for the women who told us that they had insufficient milk. The relationship that we observed was sufficiently strong and sufficiently unprecedented that we decided that we would do a study elsewhere in the world where the levels were higher.




Epidemiologically, you can get the same bang for the dose response estimation buck by going to some place where the distribution of the levels are higher.

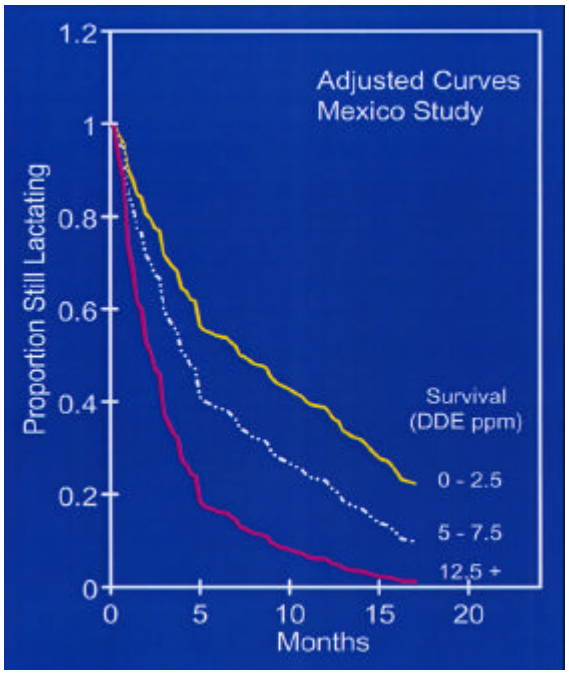
A lot of our inference depended on the women up in the top level in North Carolina and by going where the levels were higher, we could do a smaller study and get the same epidemiologic power.  We elected to do the study in Mexico




where there were quite high levels and which had the extra advantage of being only a couple of hours away by plane.

Tlahualilo is a small town in an agricultural region in Northern Mexico. Everybody was engaged in the growing of cotton. When we went there, there were cotton bolls stuck to the barbed wire and there were cotton bolls on the street.

The Mexican authorities already knew that there were pretty high levels of DDT in Tlahualilo. We would get the same estimating power from a study of 230 women in Mexico that we got from our 800+ in North Carolina.




We did basically a population based, birth cohort study for about one year of births, attempting to enroll all pregnant women who planned to stay in the area during the first 18 months of the baby's life. We enrolled 230 and followed 196 through weaning. There were 22 of them still breast feeding at 18 months.


We split women into those with interrupted lactation: mom taking medication, mom ill, hormonal contraception, mechanical problems with the breast, return to work, or mom being persuaded by herself or usually her mom to quit breast feeding that kid.

If we look at survival curves as a means of estimating the relationship between continuing to lactate and level of DDE,

MEDIAN AGE AT WEANING BY DDE
NORTH CAROLINA VS MEXICO

| DDE (ppm) | MEXICO N | MEXICO Median age (months) | NC Median age (months) | NC N |
|---|---|---|---|---|
| 0 - 2.4 | 29 | 7.5 | 7.6 | 392 |
| 2.5 - 4.9 | 59 | 5.0 | 6.0 | 282 |
| 5.0 - 7.4 | 66 | 3.0 | 3.5 | 45 |
| 7.5 - 9.9 | 33 | 3.5 | 2.2 | 18 |
| 10.0 - 12.4 | 21 | 4.0 | 2.8 | 9 |
| 12.5 + | 21 | 3.0 | 7.7 | 6 |
| Total | 229 | 5.0 | 6.2 | 752 |

we see that the women with the lowest DDE levels at any time point are always more likely to still be lactating than the women at higher levels. If you compare the exposure distribution with that in NC, you see that in North Carolina, most of the women, 392, are below 2.5 ppm DDE in milk fat. Only 29, or 10%, of the women form Mexico are that low. Conversely, about 10 percent of the women in Mexico are above 12.5 ppm, whereas, only six out of 750 or less than one percent of the North Carolina women are. In epidemiologic terms, we got unusually good coherence in the dose-response from the two studies. For example, the women with the lowest level wean at 7.5 months in North Carolina and in 7.6 months in Mexico.

Now the impact of duration of lactation in the United States in terms of mortality is perhaps $10^{-5}$ in terms of the observed difference in mortality in the first two years between breast fed and bottle fed kids. Even that is attributable to the association between breast feeding and sudden infant death syndrome. However, in most of the world where there isn't adequate clean water to make formula, nor money to pay for

enough calories, duration of lactation is the single most powerful determinant of infant mortality.

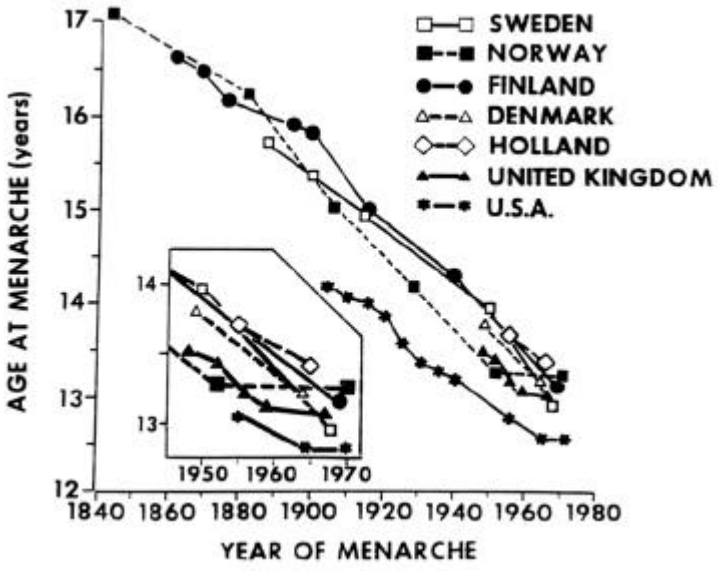
We don't see any effect on duration of lactation from PCB levels in North Carolina. We see it only with DDE. There are PCBs that can occupy estrogen receptors. This has not been confirmed by other investigators. There's a couple of people looking at it in Wisconsin, Hawaii and upstate New York. So far, no one's come up with confirmation of this. There is no animal model. Duration of lactation is very difficult to study in the laboratory because they cull and then they wean. There is no such thing really as natural duration of lactation in a rodent colony.

So we think we have some evidence that DDE at levels encountered in the US population in the late 1970s might be hormonally active. I am going to shift now to another line of evidence that points to the same thing, albeit less directly.

Menarche in white girls has been occurring earlier.

(slide 15)

Menarche has been of interest from a gynecological and public health point of view for a long, long time. We have data on menarche -- the onset of menstrual bleeding -- from the 1840s. The age of first bleeding has been going down in White girls of Northern



European extraction since 1840.

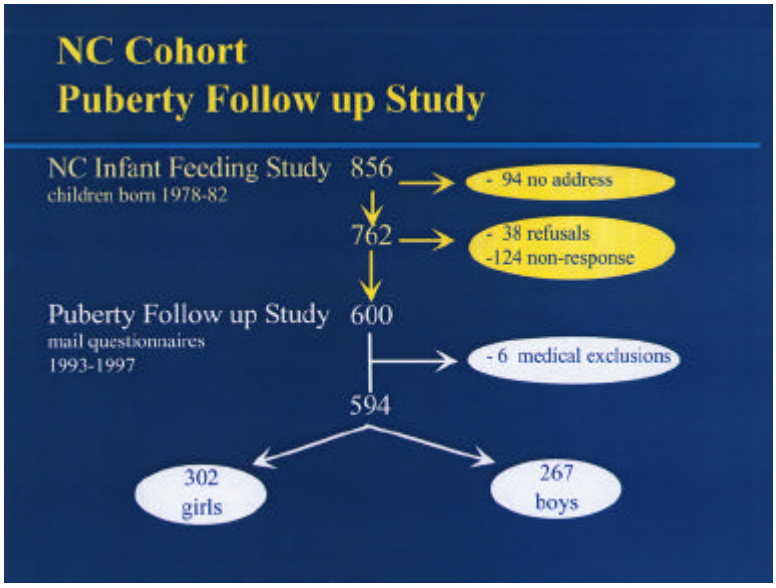
In the US, that age is median 12.7, which it got down to in the 1950s and has been since.

There aren't any secular trend data on girls of color. Marcia Herman Giddens published a paper in the Journal of Pediatrics a year ago. In it, she looked for the first time at Tanner stages -- which I'm going to show you in a second -- in girls of color. Those girls had signs of puberty much younger than had been described in white girls.

This caused a revision in the American Academy of Pediatrics guidelines on the diagnosis of precocious puberty. It wasn't because black girls were maturing earlier than they had been before, it's because normative data on girls of color had not been part of the data set on consideration of puberty.

So while there was not a direct hypothesis involving environmental chemicals and puberty, there was evidence that menarche at least was variable, we knew that the chemicals were active in the laboratory if given to the dam and the offspring observed for sexual maturation, and we had the opportunity to collect the data.

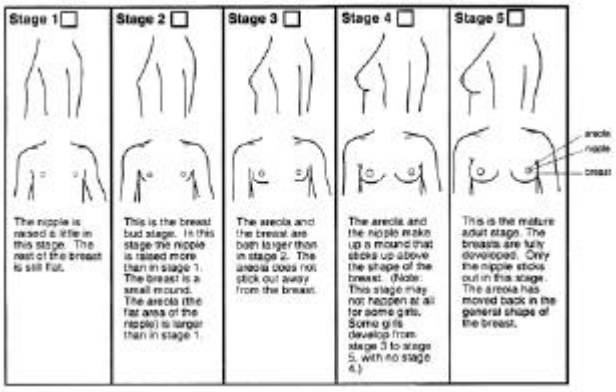
The North Carolina infant feeding study, the same study we talked about in the beginning, had 856 kids born between 1978 and 1982. When we tried to find them again in 1993 through 1997, we could find 762. Of those, 600 were still interested in participating in the study. There were six medical exclusions, and so we ended up with data on 594

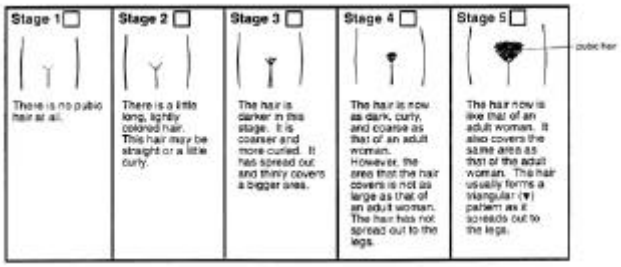


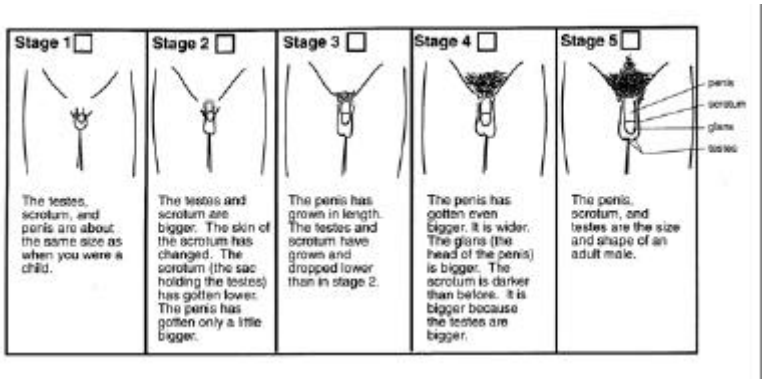

Every year, we sent them a package. The package had a little questionnaire that said, "We want to know your height. You are supposed to stand barefoot against a wall and measure your tallest." We sent them a tape measure that was tall enough so that we were sure they could use it.

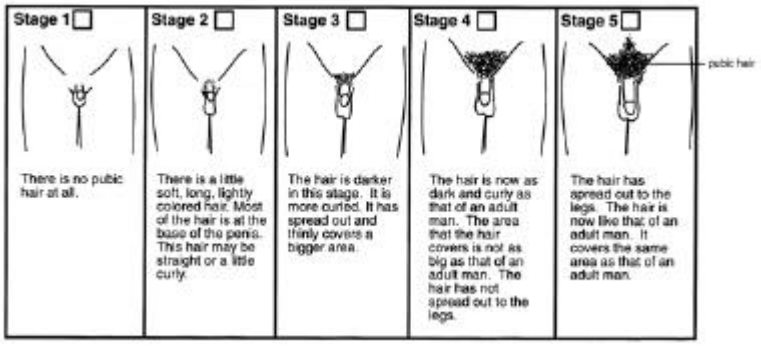

We just asked them to give us their weight.  That works really well for those of you who know the weight literature in epidemiology.

We sent them modified Tanner scales

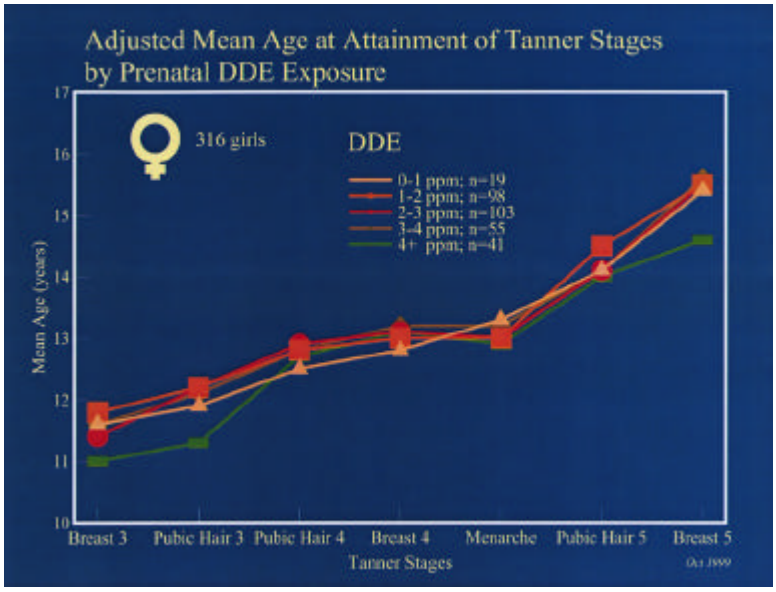



Stage 1
There is no pubic hair at all.
Stage 2
There is a little long, lightly colored hair. This hair may be straight or a little curly.
Stage 3
The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a bigger area.
Stage 4
The hair is now as dark, curly, and coarse as that of an adult woman. However, the area that the hair covers is not as large as that of an adult woman. The hair has not spread out to the legs.
Stage 5
pubic hair
The hair now is like that of an adult woman. It also covers the same area as that of the adult woman. The hair usually forms a triangular (▽) pattern as it spreads out to the legs.

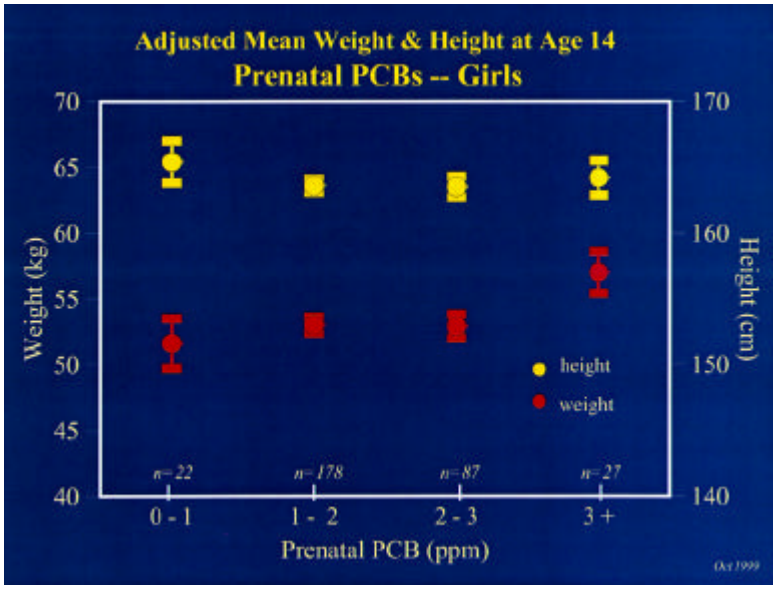

Stage 1
The testes, scrotum, and penis are about the same size as when you were a child.
Stage 2
The testes and scrotum are bigger. The skin of the scrotum has changed. The scrotum (the sac holding the testes) has gotten lower. The penis has gotten only a little bigger.
Stage 3
The penis has grown in length. The testes and scrotum have grown and dropped lower than in stage 2.
Stage 4
The penis has gotten even bigger. It is wider. The glans (the head of the penis) is bigger. The scrotum is darker than before. It is bigger because the testes are bigger.
Stage 5
penis
scrotum
glans
testes
The penis, scrotum, and testes are the size and shape of an adult male.



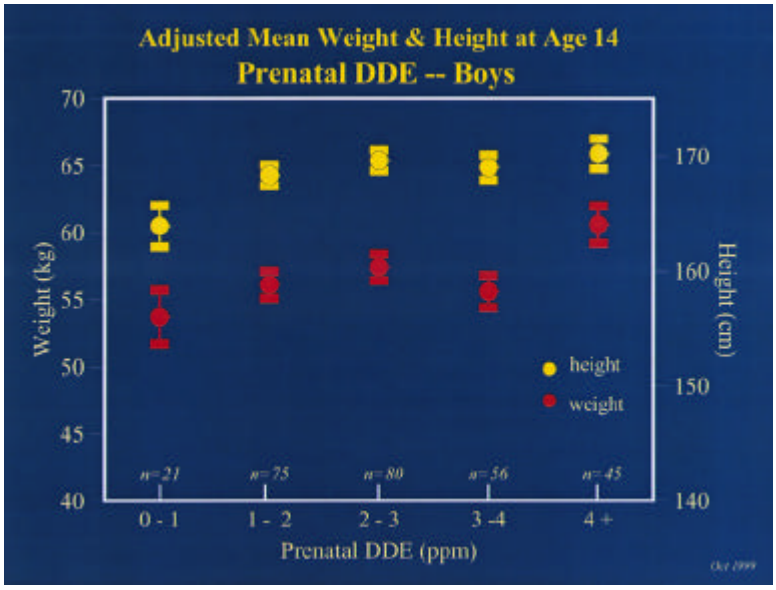

The true Tanner scales are photographs in which secondary sexual characteristics are depicted in five ordered progressive stages. The original version required an examination by a physician. We used a self-administered line drawn version. There's very good evidence that the kids can fill out these forms accurately compared to a physician exam. If we look at weight at age 14 by prenatal DDE exposure in boys, we see that those with the greatest exposures are taller and heavier - 52 kilos at the lowest exposures and 60 kilos at the highest.



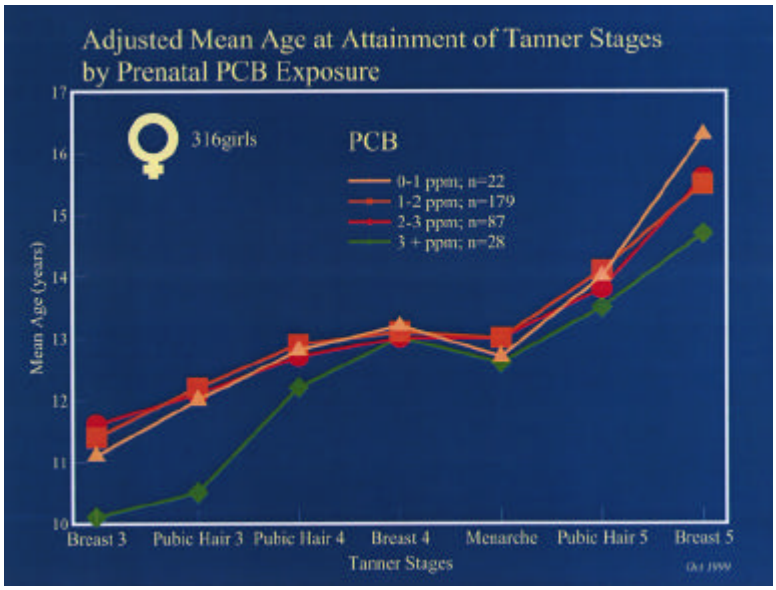

There is no effect in the boys from PCB exposure. There is no effect in either sex from the later, the later, but greater post-natal exposure from breast milk.



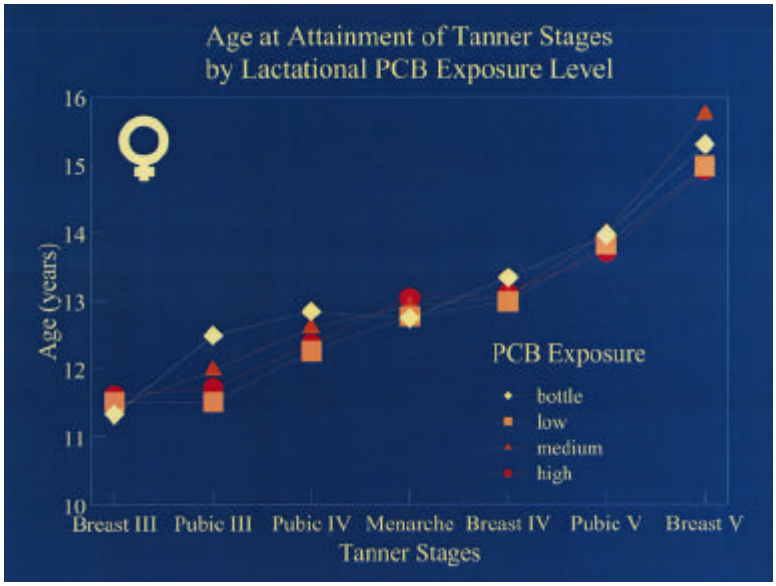
In the girls, we saw no effect on height at age 14, but see an effect on weight, but only in the highest group, and only in white girls.




We also observed that the girls with the highest pre-natal PCB exposure -- these are the girls who are heavier -- went through the earlier stages of puberty sooner by about 11 months, but they folded in later.




We had no hypothesis that individual stages of puberty would be affected. So we observe that these curves start out slightly different, but overall the process proceeds similarly. So we think that the boys with the higher pre-natal DDE exposure are taller and heavier. The white girls with the higher pre-natal PCB exposure are fatter. There does not seem to be an effect on Tanner stages on onset of puberty from either chemical in either sex




This talk is based on three papers:

Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen JD, Tingelstad J, and Tully M. PCBs and DDE in human milk: effects on growth, morbidity, and duration of lactation. Am J Public Health 77: 1294-1297, 1987

Gladen BC, Rogan WJ. DDE and shortened lactation in a northern Mexican town. Am J Pub Health, 85:504-8, 1995

Gladen BC, Ragan NB, Rogan WJ. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlordiphenyl dichloroethene. J Pediatr 136: 490-496, 2000

ANDREW SALMON: Thank you very much.
Are there any questions that anybody would like to put to Dr. Rogan at this stage?

LUCY ANDERSON: A number of years ago, we treated mice with PCBs and found a very significant increase in the reproductive efficacy of these male mice. They got more females pregnant in a shorter period of time with more babies.
I'm wondering if you had any indications of testosterone-related behaviors or anything like that in these kids to this point.

DR. ROGAN: No. The only thing we could say about the boys is that they are bigger boys. I don't know the relationship between bigness and reproductive efficacy in rodents. It wouldn't surprise me if there was such a relationship in here.
We didn't ask about any kind of behavior at age 14. When we studied them when they were in 4th grade, we did have data on conduct. It's report card derived data. We didn't see any relationship in a data set that was sensitive enough to find a first born effect on IQ and sib effect and maternal age on motor improvement and a bunch of other sort of normative things.

DR. DEMPSEY: Very entertaining talk. I'm sure it's in your articles, but can the exposure to the DDE actually be a marker for an exposure to something else and, specifically, is it possible to obtain this compound through cigarette smoking or some other major thing that affects growth -- especially something like cigarette smoking that affects lactation?

DR. ROGAN: Cigarette smoking itself shortens duration of lactation. We, actually, have data on cigarette smoking and cigarette smoking does not increase your PCB level or your DDE level.

The only thing that does increase your PCB level in our data, besides age, is -- we don't have much of a range to study it -- alcohol consumption in women who drink have higher levels than women who don't.
The DDE data are essentially disjoint for Blacks and Whites. Black women have much higher levels. We don't have many of them. They go up with age. They all go down not with parity, but with previous lactations.
We don't know of any smoking gun confounder.

FROM THE AUDIENCE: It would appear that the logic in your data is that the clear effect on breast feeding would be because the weak estrogen is present when natural estrogen levels are low, but that the lack of correlation to DDE effect on puberty is because during the pre-natal period -- I'm just guessing -- but that the xeno estrogen levels in utero are higher.
I'm asking what is the xeno estrogen effect? Is that the way to read your data?

DR. ROGAN: It would be, except there's an effect from the DDE in boys. To go the next level down, p,p-DDE which is actually the prominent species, is an anti-androgen. That shouldn't make your boys bigger – that is not what an anti-androgen should do. But t this is how it came out and somebody else is going to have to figure out if it comes out that way in their data.